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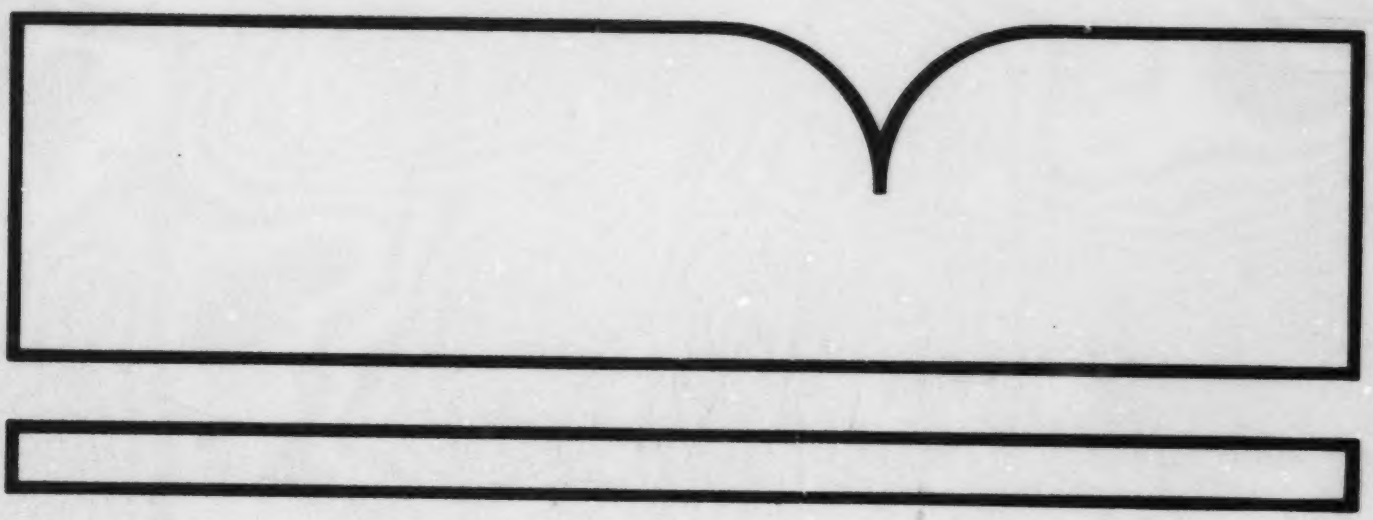

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MICROBIAL TRANSFORMATION RATES OF AZO AND TRIPHENYLMETHANE DYES

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Abstract—Using field-collected and laboratory cultures, bacterial transformation rates for one azo and two triphenylmethane dyes were studied as a means of assessing the potential degradation of these compounds in aquatic environments. Transformation was measured as loss of color by spectrophotometry, and first- and second-order transformation rate coefficients were determined. Second- order coefficients (k_b) were based on total colony-forming units (CFU) of bacteria in batch cultures. Percentages of total bacteria that transformed the dyes were determined by isolating bacterial colonies from field-collected samples and testing the isolates for the capacity to transform the dyes in dilute nutrient medium. The azo dye, Acid Orange 6, was not transformed under the aerobic conditions of these studies. Basic Violet 1 was transformed and k_1 values ranged from -0.003 to -0.023 h^{-1}, with a mean k_b of 3.5×10^{-14} L CFU^{-1} h^{-1}. Basic Violet 3 also was transformed and k_1 values ranged from -0.002 to -0.012 h^{-1}, with a mean k_b of 2.8×10^{-14} L CFU^{-1} h^{-1}. The percentage of transformer bacteria for Basic Violet 1 ranged from 6 to 28%, with a mean of 13.5%, and the range for Basic Violet 3 was 0 to 24%, with a mean of 8.32%. Therefore, the triphenylmethane dyes were transformed at similar rates, and similar fractions of the total bacterial populations were involved in the transformation.

Keywords—Azo dyes Triphenylmethane dyes Transformation rates Microbial populations

INTRODUCTION

Azo and triphenylmethane dyes are used extensively in the textile, food and cosmetic industries. Because wastewater treatment systems generally do not remove all of the dyes, wastes from these industries and from dye manufacturers commonly result in pollution of aquatic systems. Most textile dyestuffs do not exhibit significant toxicity to aquatic microbial populations [1-4]; however, some of the triphenylmethane dyes do show relatively high toxicities [5]. Such toxicity could cause a reduction in the concentrations of heterotrophic bacterial populations, thereby decreasing the overall mineralization of organic compounds, including degradation of the dyes.

Reports in the literature indicate little or no transformation of azo dyes under aerobic conditions [6], although reductive cleavage of the azo linkage is well documented [7-10]. Much less information is available regarding the triphenylmethane dyes, but published data suggest that transformation of these dyes is minimal [10].

In this study, we determined microbial transformation rate coefficients for selected dyes using both laboratory and field-collected samples. These coefficients can be used in mathematical models for predicting transformation rates in aquatic systems and can give some idea of the relative persistence of the dyes when compared with transformation rate coefficients of other organic chemicals.

MATERIALS AND METHODS

Microorganisms

Water samples were obtained from four North Georgia sites: a river, two sites in a large lake (Clark's Bridge and Balus Creek Park) and a small

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eutrophic pond. *Bacillus cereus* (ATCC 14579) and *Escherichia coli* (ATCC 23226) were obtained from the American Type Culture Collection.

Dyes

Basic Violet 1 (CI 42535), Basic Violet 3 (CI 42555) and Acid Orange 6 (CI 14270) were obtained from Aldrich Chemical Company and were 85, 95 and 85% pure, respectively. The structures of these dyes are shown in Figure 1.

Procedures

Bacterial numbers in field-collected water samples and laboratory cultures were estimated by colony counts on Standard Methods Agar incubated at 25°C. Nutrient broth (Difco) was spiked into water samples to a concentration of 0.1% and the samples were incubated on a shaker for 24 h at 20°C. Subsequent to this incubation period, dye was added at an initial concentration of 5 mg L^{-1} in each batch culture. Loss of dye during an additional incubation period of up to 300 h was measured spectrophotometrically using an LKB Ultraspec 4050 and measuring the loss in the visible spectrum. Numbers of transformers were estimated by isolating 40 to 50 colonies at random from the Standard Methods Agar plates used for enumerating the population and inoculating these isolates into 0.1% nutrient broth plus 5 mg L^{-1} dye. The addition of dilute nutrient broth to field-collected samples did not cause a significant change in the percentages of transformers, i.e., did not tend to bloom out nontransformer bacteria. First- and second-order transformation rate coefficients (k_1, k_b) were determined using procedures described by Paris et al. [11]. Transformation rates could only be determined between one and two half-lives of the dye. Dyes that sorbed to bacterial cells were extracted from pellets of centrifuged cells using reagent-grade acetone.

RESULTS

The loss of Basic Violet dyes 1 and 3 over time followed the four phases shown in Figure 2. The sorption phase lasted from 8 to 24 h and has been previously described [5]. During the sorption phase, dyes could be quantitatively extracted from bacterial cells using acetone. The sorption phase was followed by a slow transformation phase that lasted from approximately 24 to 120 h. This phase was characterized by decolorization of the dyes and an inability to recover all of the dye from bacterial cells using acetone extraction. This transformation phase was not evident in earlier experiments [5] in which attempts at quantitative acetone extraction of the dye were not made.

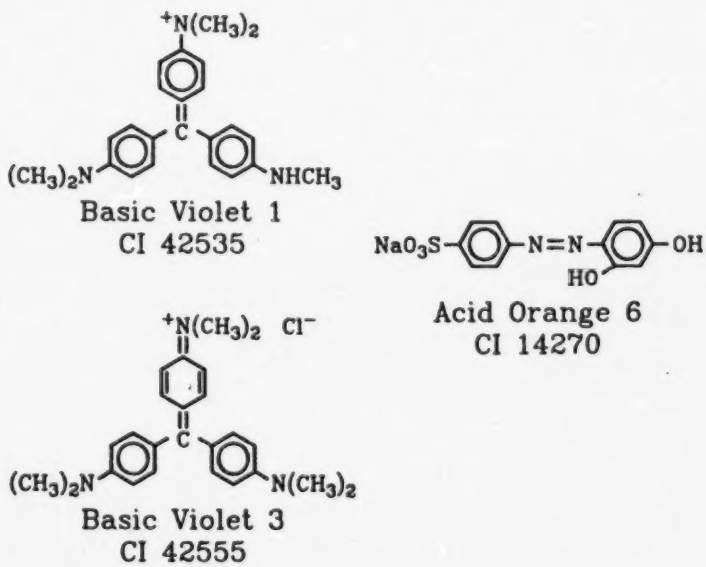

Fig. 1. Structural formulas and color index numbers (CI) for the azo and triphenylmethane dyes used in the study.

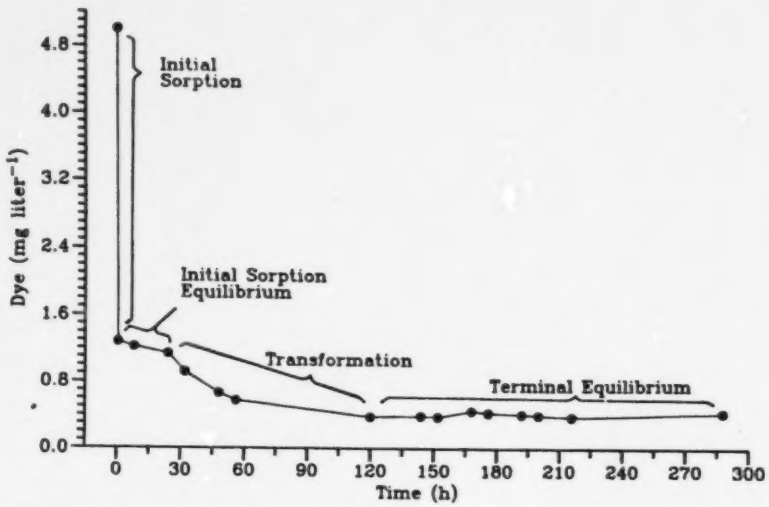

Fig. 2. Loss of Basic Violet 1 over time in a Balus Pond sample, showing the four phases typically observed in batch bacterial cultures.

Moreover, this transformation phase probably accounts for the higher sorption distribution coefficients reported for viable cells as compared with killed cells. After incubation for 120 to 150 h, a terminal equilibrium was reached in which no further loss of dye occurred.

No measurable transformation occurred with the azo dye, Acid Orange 6. Measurable transformation rates occurred in all of the 78 batch cultures tested using Basic Violet 1 (Table 1). The bacterial concentrations in batch cultures using field samples enriched with nutrient broth ranged from 5.4×10^{10} to 1.3×10^{12} CFU L^{-1}; k_1 ranged from -0.003 to -0.023 h^{-1}. The mean k_b was 3.5×10^{-14} L CFU^{-1} h^{-1}, with a range of 4.0×10^{-15} to 1.3×10^{-13} L CFU^{-1} h^{-1} and a coefficient of variation of $\pm 108\%$. For laboratory cultures, the bacterial concentrations ranged from 4.5×10^{12} to 3.0×10^{13} CFU L^{-1}; k_1 ranged from -0.002 to 0.018 h^{-1}. The mean k_b was 1.4×10^{-15} L CFU^{-1} h^{-1}, with a range of 2.0×10^{-16} to 4.0×10^{-15} L CFU-1 h^{-1} and a coefficient of variation of $\pm 109\%$.

Measurable transformation rates occurred in all of the 42 batch cultures tested using Basic Violet 3 (Table 2). Bacterial concentrations ranged from 4.6×10^{10} to 1.5×10^{12} CFU L^{-1}; k_1 ranged from -0.002 to -0.012 h^{-1}. The mean k_b was 2.7×10^{-14} L CFU^{-1} h^{-1}, with a range of 1.6×10^{-15} to 2.6×10^{-13} L CFU^{-1} h^{-1} and a coefficient of variation of $\pm 286\%$.

Among field-collected samples, the percentage of transformers (Table 3) ranged from 6 to 28%, with a mean of 13.5% for Basic Violet 1. The differences found among sites were no greater than those on different dates throughout the year at the same site. For Basic Violet 3, the values ranged from 0 to 24%, with a mean of 8.32%. Samples from the eutrophic pond consistently had greater fractions of transformer bacteria as compared with other sites.

DISCUSSION

The lack of bacterial transformation of the azo dye, Acid Orange 6, was consistent with literature reports that reductive cleavage of the azo linkage can occur [7-10] but aerobic transformation is very limited [6]. Because reductive cleavage of azo dyes is suggested to be nonenzymatic, involving reduced flavin nucleotides [9], anaerobic transformation may occur in microanaerobic zones of aufwuchs or within aggregates in aerobic systems where sufficient reducing conditions exist.

Under similar conditions, the triphenylmethane dyes were transformed at similar rates. Also, similar fractions of total bacterial populations in field-collected samples were involved in the transformation. Although Basic Violet 1 and Basic Violet 3

Table 1. Transformation rates of Basic Violet 1 in batch cultures

Site of culture	CFU L^{-1} ($\times 10^{11}$)	k_1 (h^{-1})	n	k_b (L CFU $^{-1}$ h^{-1}; $\times 10^{-14}$)
Pond	6.3 ± 0.19	0.013 ± 0.001	2	2.0 ± 0.09
	7.6 ± 0.30	0.005 ± 0.001	4	0.70 ± 0.18
	4.4 ± 0.66	0.004 ± 0.001	3	0.96 ± 0.32
	7.2 ± 0.65	0.012 ± 0.001	6	1.6 ± 0.13
	8.9 ± 0.44	0.012 ± 0.006	6	1.3 ± 0.63
	13.0 ± 0.52	0.017 ± 0.008	2	1.4 ± 0.62
	8.3 ± 0.41	0.022 ± 0.004	4	2.7 ± 0.54
	10.3 ± 0.82	0.009 ± 0.005	4	2.8 ± 3.9
Balus Creek Park	5.5 ± 0.06	0.023 ± 0.013	2	4.2 ± 2.3
	4.7 ± 0.52	0.003 ± 0.002	4	0.60 ± 0.38
	2.08 ± 0.15	0.010 ± 0.002	6	4.7 ± 0.90
	5.6 ± 0.28	0.009 ± 0.001	6	1.6 ± 0.17
	11.0 ± 0.66	0.017 ± 0.001	2	1.5 ± 0.14
	8.7 ± 0.35	0.010 ± 0.006	3	1.1 ± 0.74
	4.9 ± 0.30	0.013 ± 0.001	2	2.6 ± 0.14
	0.91 ± 0.05	0.011 ± 0.003	2	13.0 ± 2.83
Clark's Bridge	1.3 ± 0.04	0.012 ± 0.005	4	9.5 ± 3.9
	4.0 ± 0.24	0.007 ± 0.003	2	1.6 ± 0.78
	0.54 ± 0.05	0.007 ± 0.000	2	12.0 ± 0.71
Laboratory	45.0 ± 4.9	0.018 ± 0.003	4	0.4 ± 0.06
Escherichia coli	58.0 ± 4.06	0.015 ± 0.001	2	0.25 ± 0.02
	50.0 ± 4.00	0.004 ± 0.000	2	0.09 ± 0.001
Bacillus cereus	91.0 ± 5.46	0.002 ± 0.002	2	0.02 ± 0.02
	300.0 ± —	0.005 ± 0.001	2	0.02 ± 0.001
	80.0 ± 6.4	0.006 ± 0.000	2	0.07 ± 0.002

CFU, colony-forming units; k_1, first-order transformation rate coefficient; n, number of batch cultures; k_b, second-order rate coefficient. k_b range, 0.40 to 13.0; mean k_b ± C.V., 3.5 ± 108%.

can be transformed by aquatic populations, the rates of tranformation appear to be slow compared with those for many textile dyes [2]. Neither of the dyes showed degradation to undetectable limits, but they did exhibit a phase of terminal equilibrium during which no further dye loss occurred. Similar results have been observed with commercial textile dye preparations [2]. This ter-

Table 2. Transformation rates of Basic Violet 3 in batch cultures

Site of culture	CFU L^{-1} ($\times 10^{11}$)	k_1 (h^{-1})	n	k_b (L CFU $^{-1}$ h^{-1}; $\times 10^{-14}$)
Pond	12.0 ± 0.48	0.011 ± 0.001	4	0.9 ± 0.10
	15.0 ± 0.45	0.011 ± 0.005	4	0.73 ± 0.31
Balus Creek	0.46 ± 0.04	0.012 ± 0.003	4	26.0 ± 5.7
	8.6 ± 0.34	0.004 ± 0.001	4	0.42 ± 0.06
	11.0 ± 0.55	0.003 ± 0.002	4	0.27 ± 0.20
	12.0 ± 0.48	0.002 ± 0.000	4	0.18 ± 0.003
	13.0 ± 0.39	0.002 ± 0.001	4	0.20 ± 0.005
Clark's Bridge	8.3 ± 0.33	0.003 ± 0.001	4	0.31 ± 0.11
	10.0 ± 0.50	0.002 ± 0.000	4	0.16 ± 0.015
Chestatee River	7.0 ± 0.49	0.004 ± 0.0008	3	0.51 ± 0.12
	12.0 ± 0.48	0.003 ± 0.001	3	0.28 ± 0.08

CFU, colony-forming units; k_1, first-order transformation rate coefficient; n, number of batch cultures; k_b, second-order rate coefficient. k_b range, 0.16 to 26.0; mean k_b ± C.V., 2.7 ± 286%.

Table 3. Percentage of total bacteria capable of transforming the dyes in dilute nutrient broth

Site	Date	% transformers
Basic Violet 1		
Pond	Feb 7	6.0
	Feb 22	8.0
	Apr 10	28.0
	Apr 23	15.0
Balus Creek	Jan 20	10.6
	Jan 31	6.3
	Apr 3	14.0
Clark's Bridge	Feb 15	12.0
	Feb 29	22.0
Basic Violet 3		
Pond	Mar 6	24.0
	Apr 17	0.0
	Apr 30	0.0
	May 7	17.0
	Jul 30	13.0
	Sep 4	6.0
Balus Creek	Mar 13	14.6
	Jun 11	5.0
	Jul 17	4.0
	Jul 23	0.0
	Sep 18	2.0
Clark's Bridge	Mar 20	0.0
	Mar 27	0.0
	Jun 25	0.0
	Aug 13	2.0
	Oct 2	0.0
Chestatee River	Jul 10	0.0
	Aug 20	4.0
	Sep 25	0.0

minal equilibrium may have resulted from depletion of nutrients or accumulation of toxic metabolites that inhibited further microbial activity. We do not, however, expect transformation in the field to be limited by such factors. In the case of certain textile dyes, further degradation is promoted by the addition of small quantities of yeast extract, suggesting that nutrient depletion may be involved (Michaels, unpublished data).

The spacial and temporal variability in percentages of transformer bacteria that we observed among field sites is most probably a result of fluctuations in microbial community structures. Patchiness in the distribution of bacteria in lakes [12,13] and variations in the heterotrophic populations of flowing waters [14,15] are well documented, as are population changes resulting from changes in nutrients [16]. Furthermore, for environmental samples, first-order transformation rate coefficients were almost as consistent among samples as were second-order coefficients. This result indicates that the samples contained similar concentrations or activities of bacteria capable of transforming the dyes. The implication of this result is that first-order models should be about as useful as second-order models for predicting the transformation rates of these compounds.

The data presented here and those reported by other researchers suggest that the dyes appear to be slowly degraded by aerobic aquatic microbial populations. The toxicity of these persistent dyes, therefore, may decrease the overall mineralization of organic compounds by causing a reduction in concentrations and species diversity of heterotrophic bacterial communities unless they are rapidly degraded by anaerobic populations.

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